Background

Photoimmunotherapy (PIT) is an investigational anticancer treatment platform that combines cell surface binding of an antibody conjugated to a light activatable dye (IRDye® 700DX, IR700) with red-light illumination for selective cell killing. The therapeutic efficacy of photoimmunotherapy depends partly on the successful delivery of antibody conjugate to the tumor. Thus, there is a critical need to accurately quantify drug penetration, distribution, and target-cell binding at the tumor for optimal therapeutic efficacy. Current methods for drug quantification lack the cellular-level interaction of drug and target cell, providing only pixel-level information, therefore novel methods for drug quantification are needed.

Methods

We have developed a cellular-level drug quantification pipeline based on multiplex immunofluorescence imaging to measure the amount of ASP-1929 (cetuximab-IR700) that reaches the tumor site and binds the epidermal growth factor receptor (EGFR). Following an automatic image quality check to filter out imaging artifacts such as saturation, folds, and blurs, we segmented the tumor regions using a tumor detection model based on a data-driven, defined, empirical threshold. Within the tumor regions, individual cells were detected using a watershed cell segmentation method and then classified into four phenotypes based on EGFR and ASP-1929 positivity. The method for cell classification uses machine learning models that have been trained and validated. We then quantified the ASP-1929 cell surface drug coverage and categorized the cells into three bins: low (<10%), moderate (10–50%), and high (>50%) uptake. Finally, we reported the cell counts in each bin (figure 1).

Results

We analyzed data from 18 head and neck squamous cell carcinoma (HNSCC) patients enrolled in the phase 1b/2 trial, ASP-1929–181 (NCT04305795), which included 48 whole-slide images taken from tumor biopsies at screening and 24 hours post ASP-1929 infusion. Our quantification methods revealed a prominent co-expression of drug with EGFR (>50% drug uptake) at the illumination time point (24h post-infusion) and a trend toward increased drug uptake over treatment cycles. Importantly, there was negligible ASP-1929 signal in pre-treatment tumors indicating very little background with this method (>30-fold increase at cycle 1 vs pre-treatment, p<0.0001).

Conclusions

We describe a novel method for the quantification of fluorescence-conjugated therapeutics using a generalized machine learning-based framework. Using this method, we show good tumor penetration and target binding of ASP-1929 in patients with HNSCC enrolled in ASP-1929–181. Future studies will use this method for optimizing drug dose as it relates to therapeutic efficacy in both preclinical studies and clinical trials.

Trial Registration

https://classic.clinicaltrials.gov/ct2/show/NCT04305795

REFERENCES


Ethics Approval

This study obtained ethics approval by Inteview IRB for protocol ASP-1929–181. Patients gave informed consent before taking part.

Abstract 1307 Figure 1

Visualization of ASP-1929 drug penetration at a tumor site. The top row is a biopsy of a patient taken before treatment and the bottom row is a biopsy of a patient taken 24 hours post-drug infusion. As expected, in the screening biopsy all cells have negligible drug uptake whereas in the post-treatment biopsy most of the cells have high drug uptake.